ORALLY ADMINISTERED TESTOSTERONE UNDECANOATE LOADED PRONIOSOME CAPSULE FOR ENHANCEMENT OF ORAL BIOAVAILABILITY: IN VIVO PHARMACOKINETIC STUDY

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Orally Administered Testosterone Undecanoate Loaded Proniosome Capsule For Enhancement Of Oral Bioavailability: In Vivo Pharmacokinetic**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associateship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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1. Introduction

1.1 Purpose of the study

The purpose of the present study shall be to investigate potential of the proniosome to enhance the oral bioavailability of the poorly water insoluble and poorly bioavailable drug testosterone. Proniosome is known to enhance the oral bioavailability of a variety of drugs with poor bioavailability and also have been established to deliver a wide range of drugs through oral route. The present study also aims to explore potential of Testosterone loaded proniosome besides enhancing their bioavailability, reduced first-pass metabolism, reducing its dosage, restricting the off-target distribution, preventing undesired plasma peak, and larger duration of action, modulated and controlled drug release, and improving the benefit to risk ratio.

1.2 Background

1.2.1 Role of Testosterone

The prevalence of biochemical testosterone deficiency increases with age. This is partly due to decreasing testosterone levels associated with illness or debility but there is also convincing epidemiological data to show that serum free and total testosterone levels also fall with normal aging [1-2]. The symptoms of aging include tiredness, lack of energy, reduced strength, frailty, loss of libido, decreased sexual performance depression and mood change. Men with hypogonadism experience similar symptoms. This raises the question of whether some symptoms of aging could be due to relative androgen deficiency. On the other hand, similarities between normal aging and the symptoms of mild androgen deficiency make the clinical diagnosis of hypogonadism in aging men more challenging.

Epidemiological studies suggest that many significant clinical findings and important disease states are linked to low testosterone levels. These include osteoporosis [3], Alzheimer's disease [4], frailty, obesity [5] and diabetes [6].

The extent to which testosterone deficiency is involved in the pathogenesis of these conditions, or to which testosterone supplementation could be useful in their treatment is an area of great interest with many unanswered questions.

Male hypogonadism is a clinical syndrome caused by a lack of androgens or their action. Causes of hypogonadism may reflect abnormalities of the hypothalamus, pituitary, testes or target tissues. Increases in the amount of testosterone converted to estrogen under the action of the enzyme aromatase may also contribute to hypogonadism. Most aspects of the clinical syndrome are unrelated to the location of the cause. A greater factor in the production of a clinical syndrome is the age of onset. The development of hypogonadism with aging is known as late-onset hypogonadism and is characterised by loss of vitality, fatigue, loss of libido, erectile dysfunction, somnolence, depression and poor concentration. Hypogonadal ageing men also gain fat mass and lose bone mass, muscle mass and strength.

Osteoporosis refers to pathological loss of bone density and strength. It is an important condition due to its prevalence and association with bone fractures; most commonly of the hip, vertebra and forearm. A number of epidemiological studies have found that bone mineral density in the aging male population is positively associated with endogenous androgen levels [7]. Testosterone levels in young men have been shown to correlate with bone size, indicating a role in determination of peak bone mass and protection from future osteoporosis [8].

Changes in body composition are seen with aging. In general terms, aging males are prone to loss of muscle mass and a gain in fat mass, especially in the form of visceral or central fat. An epidemiological study of community dwelling men aged between 24 and 85 years has confirmed that total and free testosterone levels are inversely correlated with waist circumference and that testosterone levels are specifically related to this measure of central obesity rather than general obesity [9].

Cardiovascular disease, and its underlying pathological process atherosclerosis, is an important cause of morbidity and mortality in the developed and developing world. Coronary heart disease in particular is the commonest cause of death worldwide [10]. As

well as increasing with age, this disease is more common in the male versus female population internationally, which has led to interest in the potential role of sex hormones in modulating risk of development of atherosclerosis. Concerns about the potential adverse effects of testosterone treatment on cardiovascular disease have previously contributed to caution in prescribing testosterone to those who have, or who are at risk of, cardiovascular disease. Contrary to fears of the potential adverse effects of testosterone on cardiovascular disease, there are over forty epidemiological studies which have examined the relationship of testosterone levels to the presence or development of coronary heart disease, and none have shown a positive correlation. Many of these studies have found the presence of coronary heart disease to be associated with low testosterone levels.

Studies also show a consistent negative correlation of testosterone with blood pressure [11]. Data specific to the ageing male population suggests that this relationship is particularly powerful for systolic hypertension. Interventional trials have not found a significant effect of testosterone replacement on blood pressure [12].

The normal development of the prostate gland is dependent on the action of testosterone via the androgen receptor, and abnormal biosynthesis of the hormone or inactivating mutations of the androgen receptor are associated with a rudimentary prostate gland. Testosterone also requires conversion to dihydrotestosterone in the prostate gland for full activity. In view of this link between testosterone and prostate development, it is important to consider the impact that testosterone replacement may have on the prevalence and morbidity associated with benign prostatic hypertrophy (BPH) and prostate cancer, which are the common conditions related to pathological growth of the prostate gland.

Erectile dysfunction is a common finding in the aging male. A prevalence of over 70% was found in men older than 70 in a recent cross-sectional study [13]. Treatment with phosphodiesterase-5 (PDE-5) inhibitors is proven to be effective for the majority of men but some do not respond [14]. The condition is multi-factorial, with contributions from emotional, vascular, neurological and pharmacological factors. The concept of erectile dysfunction as a vascular disease is particularly interesting in view of the evidence

presented above, linking testosterone to atherosclerosis and describing its action as a vasodilator.

Cognitive abilities differ between males and females and these differences are present from childhood. In broad terms, girls have stronger verbal skills than boys who tend to have stronger skills related to spatial ability. It is thought that the actions of sex hormones have a role in these differences. Reviewing different cognitive strengths of male versus female humans is not within the scope of this article but the idea that cognition could be altered by testosterone deserves attention.

Mood disturbance and dysthymia are part of the clinical syndrome of hypogonadism. Epidemiological studies have found a positive association between testosterone levels and mood, and depressed aging males have lower testosterone levels than controls [15].

The aim of treatment for hypogonadism is to normalize serum testosterone levels and abolish symptoms or pathological states that are due to low testosterone levels. The exact target testosterone level is a matter of debate, but current recommendations advocate levels in the mid-lower normal adult range [16]. Truly physiological testosterone replacement would require replication of the diurnal rhythm of serum testosterone levels, but there is no current evidence that this is beneficial [16].

At the present time, it is suggested that androgen replacement should take the form of natural testosterone. Some of the effects of testosterone are mediated after conversion to estrogen or dihydrotestosterone by the enzymes aromatase and 5 alpha-reductase enzymes respectively. Other effects occur independently of the traditional action of testosterone via the classical androgen receptor- for example, its action as a vasodilator via a cell membrane action as described previously. It is therefore important that the androgen used to treat hypogonadism is amenable to the action of these metabolizing enzymes and can also mediate the non-androgen receptor actions of testosterone. Use of natural testosterone ensures this and reduces the chance of non-testosterone mediated adverse effects. There are now a number of testosterone preparations which can meet these recommendations and the main factor in deciding between them is patient choice.

Currently available testosterone preparations in common use include intramuscular injections, subcutaneous pellets, buccal tablets, transdermal gels and patches. Oral testosterone is not widely used. Unmodified testosterone taken orally is largely subject to first-pass metabolism by the liver. Oral doses 100 fold greater than physiological testosterone production can be given to achieve adequate serum levels. Methyl testosterone esters have been associated with hepatotoxicity. There has been some use of testosterone undecanoate, which is an esterified derivative of testosterone that is absorbed via the lymphatic system and bypasses the liver. Unfortunately, it produces unpredictable testosterone levels and increases testosterone levels for only a short period after each oral dose [17].

Intramuscular testosterone injections were first used around fifty years ago. Commercially available preparations contain testosterone esters in an oily vehicle. Esterification is designed to retard the release of testosterone from the depot site into the blood because the half life of unmodified testosterone would be very short. For many years intramuscular preparations were the most commonly used testosterone therapy and this is still the case in some centers. Pain can occur at injection sites, but the injections are generally well tolerated and free of major side effects. Until recently, the available intramuscular injections were designed for use at a frequency of between weekly and once every four weeks. These preparations are the cheapest mode of testosterone treatment available, but often cause supraphysiological testosterone levels in the days immediately following injection and/or low trough levels prior to the next injection during which time the symptoms of hypogonadism may return [18]. More recently, a commercial preparation of testosterone undecanoate for intramuscular injection has become available. This has a much longer half-life and produces testosterone levels in the physiological range throughout each treatment cycle [19]. The usual dose frequency is once every three months. This is much more convenient for patients but does not allow prompt cessation of treatment if a contraindication to testosterone develops. The most common example of this would be prostate cancer and it has therefore been suggested that shorter acting testosterone preparations should preferably use for treating older patients. Similar

considerations apply to the use of subcutaneous implants which take the form of cylindrical pellets injected under the skin of the abdominal wall and steadily release testosterone to provide physiological testosterone levels for up to six months. Problems also include pellet extrusion and infection [20].

Transdermal preparations of testosterone utilize the fact that the skin readily absorbs steroid hormones. Initial transdermal preparations took the form of scrotal patches with testosterone loaded on to a membranous patch. Absorption from the scrotal skin was particularly good and physiological levels of testosterone with diurnal variation were reliably attained. The scrotal patches are now rarely used because they require regular shaving or clipping of scrotal hair and because they produce rather high levels of dihydrotestosterone compared to testosterone [21]. Subsequently, non-scrotal patches were developed but the absorptive capacity of non-scrotal skin is much lower, so these patches contain additional chemicals which enhance absorption. The non-scrotal skin patches produce physiological testosterone levels without supraphysiological dihydrotestosterone levels. Unfortunately, the patches produce a high rate of local skin reactions often leading to discontinuation [22]. In the last few years, transdermal testosterone gel preparations have become available. These require daily application by patients and produce steady state physiological testosterone levels within a few days in most patients. The advantages compared with testosterone patches include invisibility, reduced skin irritation and the ability to adjust dosage, but concerns about transfer to women and children on close skin contact necessitate showering after application or coverage with clothes. Another recent development is the production of adhesive tablets which are applied twice daily to the buccal mucosa on the gum above the incisor teeth. The tablets gradually release testosterone into the systemic venous circulation and steady state physiological concentrations are achieved in most patients within two days [23].

1.2.2 Proniosome

It has been reported that active pharmaceutical ingredients (API) with less than 1 mg / ml solubility in water takes up significant percentage. Problems associated with low aqueous soluble drugs include slow or low dissolution, poor membrane permeability, variable or low bioavailability and finally poor patient efficacy [24, 25]. From the last few decades, advancement in the field of novel drug delivery has led to the development of a number of approaches to settle down the issues of low aqueous solubility of API. For example, complexation, solid dispersion, micronization, use of co-solvent, colloidal particulate (e.g. microemulsion, self-emulsifying drug delivery system, liposome, niosome and solid lipid nanoparticle) and nanosizing (nanoemulsion, nanosuspension, nanoparticle, nanocrystals) [26]. Among all, lipid-based colloidal particulate such as liposome and niosome has attracted the major attention of formulation scientist as an alternative colloidal drug delivery system. This lipid-based colloidal drug delivery possess a distinct advantage over conventional dosage forms, for example, the particle can act as a drug reservoir, can carry the hydrophilic drug by encapsulation and or partitioning of hydrophobic drug into hydrophobic domain [5,6]. Modification of the particulate composition or surface properties can control the rate of drug release and/or affinity of the target site [26, 27].

To overcome the drawback associated with vesicular drug delivery system led to the development of ‘‘proniosomes’’. In this approach, two previously mentioned techniques were combined together by extending the proliposome approach to niosome through the formation of proniosomes [28-30]. Proniosomes are either non-ionic surfactant liquid crystals with a jelly-like consistency or dry, free flowing formulation of water-soluble surfactant coated carrier18–20. Both pharmaceutical dosage forms can be reconstituted by momentary agitation with hot aqueous media to form a niosomal suspension suitable for administration via oral as well as other routes [31-32]. Proniosomes can be used for entrapping both hydrophobic and hydrophilic drugs [33]. The additional merit of proniosomes compare to other vesicular drug delivery systems is low toxicity due to non-ionic nature [33]. Formulation of proniosomes does not require any special precautions and conditions. In addition, proniosomes can be prepared without the use of undesirable

solvents by a simple method for the routine and large-scale production. They also minimize problems of physical stability, fusion, leakage, sedimentation and aggregation on storage [34]. Drug entrapment in the vesicular structure of proniosomes prolongs the existence of drug in the systematic circulation, enhances the penetration in the target tissue and reduces the toxicity [35]. This review will enlighten the basic concepts and up to date research finding concerning the very promising use of proniosomes. Particularly, we address the recent progress achieved by proniosomes in the field of drug delivery system.

1.2.2.1 Material used in the preparation of Proniosome

Materials used in the formulation of both types of proniosomes includes a non-ionic surfactant, the membrane stabilizer, small amount of water and alcoholic solvent ‘‘such as absolute ethanol, propanol, isopropanol, butanol, propylene glycol or liquid polyethylene glycols’’ to formulate proniosomal gels [36]. However, to prepare dry free flowing proniosomal powders, the non-ionic surfactant, carrier material, membrane stabilizer and coating material are also required. A desirable characteristic of the carrier material used in the formulation of dry proniosomes comprises safety and non-toxicity, free flow, poor solubility in the loaded mixture solution and good water solubility for ease of hydration [37]. Carrier material used in the formulation of dry proniosomes permits the flexibility of surfactant and other components. Furthermore, it increases the surface area and encourages the efficient loading of the drug inside the proniosomes. The presence of steroids in the cell membrane provides stability to the membrane; therefore, cholesterol is mainly used as membrane stabilizer in the preparation of proniosomes [38]. The surfactant has varieties of function such as permeability enhancer, emulsifier wetting agent and solubilizers. Vehicles used in the reconstitution of proniosomes include phosphate buffer (pH 7.4), hot water and 0.1% glycerol.

1.2.2.2 Methods

Proniosomal preparation ‘‘coacervation–phase separation technique

The proniosomal can be prepared by the method reported by Vora et al. and modified by Ibrahim et al. Accurately weighed surface active agent were mixed with the appropriate amount of cholesterol in glass vials. Absolute ethanol were added to the surfactant or surfactant/cholesterol mixtures then vials were tightly sealed and warmed in a water bath (55–60°C) for 5 min while shaking until complete dissolution of lipids.

To each of the formed transparent hot lipid solutions, little amount of distilled water about 40% of the total solvent added’’, heated to 55–60°C were added to the solution while warming in the water bath for 3–5 min until a clear or translucent solution was produced. The mixtures were allowed to cool down at room temperature and observed for the formation of transparent solution, two-phase liquids, translucent, transparent or white creamy proniosomal gels.

Drug loading into proniosomal gel formulations

The drug could be added to the non-ionic surfactant/cholesterol mixture and dissolved by the aid of absolute ethanol while warming at 50–60°C in a water bath if it is lipid-soluble. However, it might be dissolved first either in the alcohol or in distilled water and added to the hot lipid solution before cooling down to gel state according to the drug physicochemical properties [16]. It is important to note that the addition of the drug did not show turbidity or precipitated crystals in liquid preparations before gelation and also it should not precipitate into the gel phase.

Free flowing proniosomal dry powder preparation

Slurry method

In this method, the slurry is prepared by using the carrier and surfactant/drug solution in a round-bottom flask. The additional organic solvent solution may be added to form slurry if lower surfactant loading occurs. The flask is attached to the rotary evaporator and vacuum

is applied until the powder appeared to be dry and free flowing. The powder should be stored in sealed containers at 4℃. The time required to produce proniosomes is independent of the ratio of surfactant solution to the carrier material and appears to be flexible [33, 39, 40].

Spray-coating method

This method involves preparation of proniosomes by spraying surfactant/drug in an organic solvent onto a carrier or coating material and then evaporating the solvent. Because the carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant loading has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves [41].

Niosome from proniosome by hydration

Niosome can be prepared by hydration of proniosomes. Here, the aqueous phase containing the drug is added to proniosomes with brief agitation at a temperature higher than the mean transition phase temperature of non-ionic surfactant used in the preparation of proniosomes.

Proniosomal oral powder formulation

This Proniosome powder can be orally administrated in different ways (viz compressed into a tablet, or filled with hard gelatin capsule shell etc.). For example, proniosomal oral tablets for controlled delivery and enhanced pharmacokinetic properties of acemetacin (AC) were reported by Shehata et al. In this study, positively charged proniosomes composed of 70:20:10 of Span 60/cholesterol (Chol)/stearylamine, respectively, were successively compressed into the tablets using direct compression method. The in vivo evaluation of the prepared proniosomes (powder or tablet forms) after oral administration was investigated by the determination of AC and its active metabolite indomethacin (IND) in the blood of albino rabbits [20]. The mean pharmacokinetic parameters of AC and IND

from different formulations shows increased $t_{1/2}$ and area under the curve (AUC) of both AC and IND from proniosomal tablets compared with both proniosomal powders and AC plain tablets20. Consistent with the above result, formulation and evaluation of granisetron provesicular buccoadhesive tablets was reported by Ahmed et al. In vivo pharmacokinetic study reveal higher bioavailability of buccal formulation relative to conventional oral formulation of granisetron (AUC0–1 is 89.97 and 38.18 ng h/ml for buccal and oral formulation, respectively)51. A significantly lower and delayed Cmax (12.09 ± 4.47 ng/ml, at 8 h) was observed after buccal application compared to conventional oral tablet (31.66 ± 10.15 ng/ml, at 0.5 h) [42]. Maltodextrin-based proniosomes of nateglinide and their potential as controlled delivery system for diabetic therapy was reported by Sahoo et al. The study revealed that the rate and extent of absorption of nateglinide from the proniosomal formulation was comparatively enhanced that of the pure drug. The nateglinide proniosome formulation exhibited significantly higher plasma concentration than those of pure drug [43]. Improved gastrointestinal (GI) permeation and neuroprotective activity of proniosomal-loaded Nigella sativa was reported by Akhtar et al. Results indicates that prepared proniosome shows smaller size with high entrapment efficiency [44]. The permeation enhancement ratio was found to be 2:16 in comparison to control [44]. The in vivo study revealed that the proniosomal dispersion significantly improves the neuroprotective activity in comparison to standard and control formulation [44].

1.2.3 Drug Profile

Name of molecule: Testosterone [45]

Name of salt: Testosterone undecanoate

Testosterone is the primary sex hormone and anabolic steroid in males. In humans, testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair.

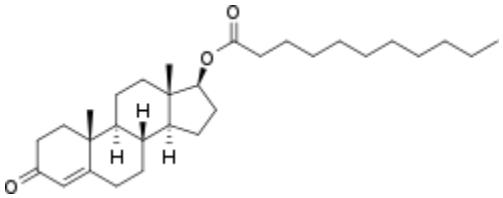

Figure 1: Chemical Structure of Testosterone undecanoate

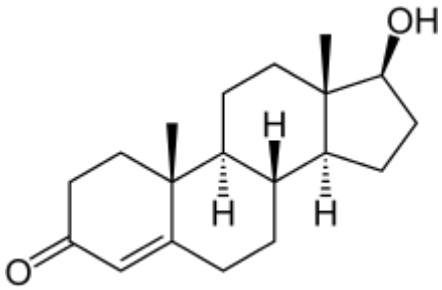

Figure 2: Chemical Structure of Testosterone

- Empirical formula: $C_{19}H_{28}O_2$ (Testosterone) $C_{30}H_{48}O_3$ (Testosterone Undecanoate)
- Molecular Weight: 456.711 gmol−1
- Bioavailability: Oral: 3–7%
- Intramuscular: high
- Protein binding: High (testosterone)
- Metabolism: Liver
- Metabolites: Testosterone, undecanoic acid, metabolites of testosterone
- Elimination half-life:
- Testosterone: 3-4 hr
- Testosterone undeconate: 20hr.
- Excretion: ~90% Urine, 6% faeces
- Melting Point: 151.0 °C

- Solubility: Testosterone is practically insoluble in water, soluble 1 g in 5 mL of ethanol and 100 mL of ether, and soluble in vegetable oils.
- Mechanism of action: testosterone and is an androgen and anabolic–androgenic steroid (AAS). That is, it is an agonist of the androgen receptor (AR).
- Use: Testosterone undecanoate is used in androgen replacement therapy. It is specifically approved only for the treatment of hypogonadism.

1.3 Factors Prompting to undertake the research project

There are various factors to undertake this research. The details of the factor are as follow:

1.3.1 Role of Testosterone in several diseases

Epidemiological studies suggest that many significant clinical findings and important disease states are linked to low testosterone levels. These include osteoporosis, Alzheimer's disease, frailty, obesity, diabetes, hypercholesterolemia, hypertension), cardiac failure and ischemic heart disease The extent to which testosterone deficiency is involved in the pathogenesis of these conditions, or to which testosterone supplementation could be useful in their treatment is an area of great interest with many unanswered questions.

1.3.2 Low Oral Bioavailability of testosterone

Testosterone is well-absorbed but extensively metabolized with oral administration due to the first pass through the intestines and liver [46-47]. It is rapidly and completely inactivated in men at doses of less than 200 mg [46]. In large doses, such as 200 mg however, significant increases in circulating testosterone levels become apparent. In addition, while a 60 mg dose has no effect on testosterone levels in men, this dose does measurably increase testosterone levels in prepubertal boys and women [46]. The oral bioavailability of testosterone in young women after a single 25 mg dose was found to be 3.6 ± 2.5% [47].

1.3.3 Limitation of conventional therapy of testosterone

Commercially available preparations contain testosterone esters in an oily vehicle. Esterifi- cation is designed to retard the release of testosterone from the depot site into the blood because the half life of unmodified testosterone would be very short. For many years intramuscular preparations were the most commonly used testosterone therapy and this is still the case in some centers. Pain can occur at injection sites.

1.3.4 Enhanced oral bioavailability through the proniosome

Proniosome enahcne the oral bioabailbilty of poorly bioavailable drug through proniosomes is due to the following two causes. First, the niosomes formed after hydration of proniosomes in gastrointestinal tract have bioadhesive property to adhere to the gastric wall proceeded by endocytosis. Second, niosomes possibly enhance the lymphatic transport that was capable of avoiding first pass effect and ultimately leads to higher bioavailability. Another cause is the use of surfactant, i.e. span 60. Surfactants have the capability to open tight junctions of intestinal epithelial wall temporarily by provoking ultra-structural changes and thereby, enhance intestinal epithelial permeability [24].

2. Review of Literature

Liang et al (2021) prepared Solid Self-Emulsifying Drug Delivery System (Solid SEDDS) for Testosterone Undecanoate. First, a TU-SEDDS was developed by using rational blends of components with the good solubilizing ability for TU. Next, a ternary phase diagram was constructed to determine the self-emulsifying region, and the formulation was optimized. Then, the solid TU-SEDDS formulation was established by screening suitable solid adsorptions. Finally, the prepared SEDDS, TUSEDDS and solid TU-SEDDS formulations were evaluated in vitro and in vivo. The size of the solid TU-SEDDS was 189.1 ± 0.23 nm. The Transmission Electron Microscopy (TEM) results showed that the oil droplets were homogenous and spherical with good integrity. The Differential Scanning Calorimetry (DSC) and X-Ray Powder Dffraction (XRD) results indicated that the solid TU-SEDDS formulation almost preserves the amorphous state. Scanning Electron Microscopy (SEM) indicated that neusilin US2 successfully adsorbed the TU-SEDDS. Drug release indicated that the dissolution of the solid TU-SEDDS was faster than that of Andriol Testocaps ®. Furthermore, in vivo pharmacokinetic (PK) studies in Sprague-Dawley (SD) rats showed that the Area Under the Curve (AUC) of the solid TU-SEDDS (487.54±208.80 μg/L×h) was higher than that of Andriol Testocaps® (418.93±273.52 μg/L×h, $P < 0.05$). In beagles not fed a high-fat diet, the AUC of the solid TU-SEDDS (5.81±4.03 μg/L×h) was higher than that of Andriol Testocaps ® (5.53±3.43 μg/L×h, $P > 0.05$). In beagles fed a high-fat diet, the AUC of the solid TUSEDDS (38.18±21.90 μg/L×h) was higher than that of Andriol Testocaps® (37.17±13.79 μg/L×h, $P > 0.05$) [48].

Shruthi et al (2020) prepared Resveratrol-loaded proniosomes. Resveratrol was encapsulated into proniosomes with the aim of enhancing its applications in food. Maltodextrin, lactose monohydrate and pullulan were used as wall materials for the preparation of resveratrol-loaded proniosomes by thin-film hydration technique. The morphological, microstructural, entrapment and release properties were determined. The wall material had marked ($p < 0.01$) influence on the shape and hydrodynamic diameter of

proniosomes. Maltodextrin-based proniosomes were smooth and spherical shaped while lactose and pullulan-based proniosomes were flaky with sharp edges. The maltodextrin-based niosomes had the lowest hydrodynamic diameter of 168.73 nm, zeta potential of − 29.6 mV, entrapment efficiency of 90.11%, and release of 21.8% at 2 h and 78.2% after 8 h in gastric and intestinal conditions, respectively. Fourier-transform infrared spectroscopy and X-ray diffractometry confirmed successful encapsulation of resveratrol. The antioxidant activity of maltodextrin and lactose-based resveratrol-loaded proniosomes was comparable to that of pure resveratrol. From dynamic light scattering, FTIR, in-vitro release, and antioxidant activity studies, it could be concluded that encapsulation of resveratrol as proniosomes would considerably enhance its bioavailability. Milk and yogurt fortified with resveratrol-loaded proniosomes did not exhibit any adverse effect on organoleptic qualities [49]

Khudair et al (2020) prepared the Letrozole-loaded nonionic surfactant vesicles prepared via a slurry-based proniosome technology. Slurry-based Letrozole (LTZ)-loaded proniosomes were designed using sucrose or sorbitol as carriers and various ratios of cholesterol (CH) and Tween 80 (T80) as lipid composition. Proniosomes were hydrated and probesonicated to generate nano-vesicles. The proniosome powders were characterized in terms of morphology using scanning electron microscopy, and drug crystallinity using differential scanning calorimetry (DSC) and X-ray diffraction (XRD). The niosomes generated from proniosomes were characterized and compared to conventional niosomes, in terms of size, zeta potential, drug entrapment, storage stability, and drug release. All formulations had size measurements in the range of 100–194 nm, polydispersity index (PDI) values below 0.3, and zeta potential values below – 23 mV. Drug entrapment was the highest for niosomes generated from sucrose-based proniosomes (CH:T80; 1:1), reaching 74% compared to less than 50% for conventional niosomes. Storage for 3 months at 4 °C resulted in minor drug leakage whilst most drug was leaked from vesicles stored at room temperature. DSC and XRD studies showed that LTZ was converted into its amorphous form upon incorporation into proniosomes. Drug release

exhibited a biphasic pattern, being fast at the first 24 h (up to 65% released) followed by a very slow release phase for a duration of one month, releasing at least 95%. The release profile of niosomes fits best with the Higuchi model. Overall, in this study, a facile approach to generating niosomes incorporating LTZ using a slurry-based proniosome technology was demonstrated. The niosomes provided high drug entrapment and controlled biphasic release over one month [50].

Hua et al (2018) prepared Nanostructured Lipid Carriers Loaded Testosterone Undecanoate. To enhance the bioavailability of testosterone undecanoate (TU) and overcome the current problem of soft capsules (Andriol Testocaps®), Nano-structured lipid carriers (NLC) for TU was developed. First, suspension of TU-loaded NLC (TU-NLC) was prepared by high pressure homogenization; then adsorbent or a protective agent β-cyclodextrin was used to solidify the suspension through a vacuum system; finally, the solid powder of TU-loaded NLC (solid TU-NLC) was filled into hard capsules. The characteristics of solid TU-NLC, were investigated in vitro and vivo. The particle size of TU-NLC was about 273.3 nm, the potential was 0.156 ± 0.04. Transmission electron microscope (TEM) revealed that the NLC was spherical and uniform. Differential scanning calorimetry (DSC) suggested the drug had been encapsulated into NLC lipid matrix. The drug release proved that solid TU-NLC showed a higher dissolution in vitro. The CaCO-2 cell permeability showed that solid TU-NLC could enhance trans-membrane absorption of the TU. Moreover, the AUC of solid TU-NLC formulations (4304±550.50μg/Lmin) was higher than commercial product Andriol Testocaps® (3075±372.50μg/Lmin) [51].

Khatoon et al (2017) explained the application of proniosome in drug delivery and drug targeting. Vesicular drug delivery systems have gained wide attention in the field of nanotechnology. Among them proniosomes become the superior over other vesicular carriers. Proniosomes are dry formulations of water soluble nonionic surfactant coated carrier system which immediately forms niosomes upon hydration. They have the

capability to overcome the instability problems associated with Niosomes and liposomes and have the potential to improve solubility, bioavailability, and absorption of various drugs. Furthermore, they offer versatile drug delivery concept for enormous number of hydrophilic and hydrophobic drugs. They have the potential to deliver drugs effectively through different routes at specific site of action to achieve controlled release action and reduce toxic effects associated with drugs. This discusses the general preparation techniques of proniosomes and mainly focus on the applications of proniosomes in drug delivery and targeting. Moreover, this review demonstrates critical appraisal of the literature for proniosomes. Additionally, this review extensively explains the potential of proniosomes in delivering drugs via different routes, such as oral, parenteral, dermal and transdermal, ocular, oral mucosal, vaginal, pulmonary, and intranasal. Finally, the comparison of proniosomes with niosomes manifests the clear distinction between them. Moreover, proniosomes need to be explored for proteins and peptide delivery and in the field of nutraceuticals and develop pilot plant scale up studies to investigate them in industrial set up [52].

Boddu et al (2017) prepared the Proniosomes of Felodipine. A lipid based drug delivery has become an emerging strategy for improved oral delivery of poorly soluble drugs. Drug encapsulation in the vesicles is one such system which helps to prolong drug duration in systemic circulation and decreases the toxicity by selective uptake. Based on this technique, a number of vesicular drug delivery systems such as liposomes, niosomes and provesicular systems like proliposomes and proniosomes have been developed. The aim of the present investigation was to develop felodipine proniosomes to enhance the solubility. Felodipine loaded proniosomes were prepared by varying ratios of span 60 and cholesterol by solvent evaporation method. Proniosomes were characterized for vesicle size, micromeritics, entrapment efficiency and dissolution behavior. Solid state behavior was evaluated by Differential scanning calorimetry (DSC), Scanning electron microscopy (SEM) and Fourier transform infrared (FTIR) studies. The formulated proniosomes showed improved dissolution characteristics which were assessed from dissolution

efficiency, mean dissolution rate data. The transformation of crystalline form of the drug to amorphous and/or molecular state was represented by solid state characterization. FTIR studies showed that absence of interaction between drug and other formulation excipients [53].

Ahmad et al (2016) explained the Technology overview and drug delivery application of proniosome. Rapid advancement in the field of colloidal science has shown a great progress in the development of proniosome technology (PT) as an impending drug delivery system. PT is a valuable carrier system for delivery of hydrophobic as well as hydrophilic drugs. It is a liquid crystalline compact niosomal hybrid, which upon hydration gives niosomes. They can augment the bioavailability of encapsulated drug and provide better therapeutic activity in a controlled manner. Non-toxicity, penetration enhancing the effect of surfactant and modified drug release from the proniosomal transdermal gel has attracted a greater attention of formulation scientist toward PT. Free flowing dry proniosomal powder are suitable for unit dosage forms such as tablet and capsules. Proniosomes are auspicious drug delivery system for the future. Proniosomes-derived niosomes are a better alternative to the other vesicular system due to their superior physicochemical stability and effective drug delivery capability. The focus of this review is to bring out all the aspects of proniosomes including their different compositions, various methods of preparation, characterization and recent development in their therapeutic applications [54].

Shehata et al (2014) Proniosomal Oral Tablets for Controlled Delivery and Enhanced Pharmacokinetic Properties of Acemetacin. Free-flowing proniosomal powders of acemetacin (AC) were prepared using the slurry method and maltodextrin as carrier. Positively charged proniosomes composed of 70:20:10 of Span 60/cholesterol (Chol)/stearylamine (SA), respectively, were successively compressed into tablets using direct compression method. The tablets were characterized for weight variability, friability, hardness, drug content uniformity, and dissolution properties. The in vivo

evaluation of the prepared proniosomes (powder or tablet forms) after oral administration was investigated by the determination of AC and its active metabolite indomethacin (IND) in the blood of albino rabbits. Results indicated that the increase of Chol from 10% to 20% markedly reduced the efflux of the drug. Further Chol addition from 30% to 50% led to increased AC release rates. The proniosome tablets of AC showed greater hardness and disintegration time and less friability than AC plain tablets. The dissolution of proniosomal tablets indicated a lower drug release percentage compared to powdered proniosomes and AC plain tablets. The mean pharmacokinetic parameters of AC and IND from different formulations indicated increased t1/2 and area under the curve (AUC) of both AC and IND for proniosomal tablets compared with both proniosomal powders and AC plain tablets. This study suggested the formulation of AC proniosomal powder into tablets to control and extend its pharmacologic effects [55].

Veerareddy et al (2012) Enhanced oral bioavailability of isradipine via proniosomal systems. Proniosomes were prepared by film deposition on carrier's method using various molar ratios of nonionic surfactants such as span20, span40, span60, and span80 with cholesterol as membrane stabilizing agent and dicetylphosphate as a charge inducer. The formation of niosomes and surface morphology of proniosome formulations were studied by optical and scanning electron microscopy (SEM), respectively. The prepared proniosomes have shown higher dissolution of isradipine compared with pure drug powder. Fourier transform infrared spectroscopy, differential scanning calorimetry, and powder X-ray diffractometry studies were performed to understand the solid state properties of the drug. Ex vivo permeation enhancement assessed from flux, permeability coefficient, and enhancement ratio were significantly higher for proniosomes compared with control. The pharmacokinetic parameters were evaluated in male albino Wistar rats and a significant enhancement in the bioavailability (2.3-fold) was observed from optimized proniosome formulation compared with control (oral suspension). The stability study reveals that the proniosome formulations are stable when stored at 4°C [56].

Muchow et al (2013) prepared the Testosterone undecanoate loaded nanostructured lipid carriers (NLC) to improve the oral bioavailbility. Testosterone undecanoate (TU) has a low oral bioavailability of about 3 % in beagle dogs and 7 % in men. To avoid administration of a single dose in two oral units and to increase lymphatic absorption to minimise side effects in the liver, TU was incorporated into nanostructured lipid carriers (NLC) and formulated as drug nanocrystals. Testosterone (T) was run as nanocrystal formulation for reasons of comparison. NLC and drug nanocrystals were produced by high pressure homogenisation. The in vivo studies were performed in male Wistar rats. The drug absorbed was quantified as testosterone analysed by EIA assay. The nanocarriers were compared with the commercial product Andriol Testocaps®. The drug nanocrystals reached about half the AUC of Andriol Testocaps® (testosterone) or were just below (testosterone undecanoate). The best NLC formulations possessed a 2 times higher AUC compared to Andriol Testocaps®. Highest bioavailability was obtained with 200 nm NLC. Nature of the lipid for the particle matrix and the drug loading (15 %, 30 %) affected distinctly the shape of the serum profile (cmax, tmax, prolongation of serum levels). Modification of these parameters should allow controlled adjustment of the serum profile to therapeutic needs. Due to the reduced volume of the nanocarriers, application of a single dose in one unit is possible [57].

Gurrapu et al (2012) Improved oral delivery of valsartan from maltodextrin based proniosome powders. The proniosome powders were prepared by varying the ratio of span 60 and cholesterol and evaluated for micromeritic properties and the results indicate acceptable flow properties. The formulation containing equimolar ratio of span 60 and cholesterol showed smaller vesicle size, high surface charge and entrapment efficiency. The formation of niosomes and surface morphology of optimized proniosome formulation was studied by optical and scanning electron microscopy, respectively. FT-IR, differential scanning calorimetry, and powder X-ray diffraction studies performed to understand the solid state properties of the drug reveal the absence of chemical interaction, drug transformation from crystalline to amorphous and molecular state. The in vitro dissolution

study carried out in both simulated gastric and intestinal fluid demonstrate improved dissolution characteristics compared to pure drug. The augment in permeation enhancement from proniosome formulation across rat intestine suggest the potential of proniosome carriers for improved oral delivery of valsartan [58].

Stanworth et al (2008) demonstrated the role of Testosterone for the aging male. An international consensus document was recently published and provides guidance on the diagnosis, treatment and monitoring of late-onset hypogonadism (LOH) in men. The diagnosis of LOH requires biochemical and clinical components. Controversy in defining the clinical syndrome continues due to the high prevalence of hypogonadal symptoms in the aging male population and the non-specific nature of these symptoms. Further controversy surrounds setting a lower limit of normal testosterone, the limitations of the commonly available total testosterone result in assessing some patients and the unavailability of reliable measures of bioavailable or free testosterone for general clinical use. As with any clinical intervention testosterone treatment should be judged on a balance of risk versus benefit. The traditional benefits of testosterone on sexual function, mood, strength and quality of life remain the primary goals of treatment but possible beneficial effects on other parameters such as bone density, obesity, insulin resistance and angina are emerging and will be reviewed. Potential concerns regarding the effects of testosterone on prostate disease, aggression and polycythaemia will also be addressed. The options available for treatment have increased in recent years with the availability of a number of testosterone preparations which can reliably produce physiological serum concentrations [59].

3. Aim and Objective

3.1 Aim

The aging population is becoming more and more, which is significantly associated with a decrease in serum testosterone levels, and causing a series of clinical signs and symptoms of hypogonadism like sexual function, infertility, fatigue, anemia, muscle strength, etc. Testosterone replacement therapy is required for patients with low testosterone levels associated with ages. However, oral testosterone preparations cause a rapidly metabolized due to its first pass through the liver, which is unable to raise serum testosterone level into the normal range. Testosterone undecanoate (TU) is a pro-drug of testosterone and absorbed in the intestinal lymphatics. What is more important, TU has been used with success in hypo-gonadal men[, for instance, Andriol and Andriol Testocaps, which are both soft capsules, the latter is a solution of TU in castor oil and Lauroglycol FCC, which takes the place of the former because the long shelf time. \ However, for Andriol Testocaps, the gastrointestinal adverse reactions are more apparent when the soft capsules are taken for long-term use. Therefore, there is a clear need for improved solid oral TU preparations to overcome the above problems, furthermore, which could increase the bioavailability of TU. The bioavailable TU is mainly absorbed via the lymphatic route. [51]

Several other formulations like Subcutaneous pellet, Trandermal Patch, Trandermal gel, Buccal tablet was prepared to improve the oral bioavailability of the testosterone undeconate but However, none of the previously reported studies gave the satisfactory results [58].

The use of phospholipid vesicles (liposomes) as drug carriers has several disadvantages due to physical instability and oxidative or hydrolysis degradation of the phospholipids. This pharmaceutical problem requires special handling and storage conditions, which increase the cost and make it impossible to scale up and manufacture liposomal formulations except for highly important drugs used in tumor targeting. Unlike liposomes, niosomal vesicles can survive the gastrointestinal acidic, bile, and enzymatic conditions.

However, niosomal dispersion also suffers physical instability such as vesicle aggregations, fusion, size changes, and drug leaks to the outside buffer medium. A possible solution for the defect in physical stability is the proniosome strategy [55].

The proniosome tactic ameliorates these problems by using dry, free-flowing product, which is more stable during sterilization and storage. Ease of distribution, transfer, measuring and storage make proniosome a versatile delivery system. Several studies have been reported which prove the utility of oral proniosomal powders in providing the enhanced solubility and bioavailability for poorly soluble drugs [58].

Proniosome enhance the oral bioavailability of poorly bioavailable drug through proniosomes is due to the following two causes. First, the niosomes formed after hydration of proniosomes in gastrointestinal tract have bioadhesive property to adhere to the gastric wall proceeded by endocytosis. Second, niosomes possibly enhance the lymphatic transport that was capable of avoiding first pass effect and ultimately leads to higher bioavailability. Another cause is the use of surfactant, i.e. span 60. Surfactants have the capability to open tight junctions of intestinal epithelial wall temporarily by provoking ultra-structural changes and thereby, enhance intestinal epithelial permeability [24].

In the recent past, several strategies have been adopted to enhance the oral bioavailability of testosterone undecanoate However; no relevant studies about Testosterone undecanoate proniosomal powders for oral administration have been reported yet. Henceforth, the present systematic study encompasses the formulation and characterization of Testosterone undecanoate loaded proniosomal powders capsule to enhance the oral bioavailability.

3.2 Objective

- To sustained the release of the drug
- To improve the oral bioavailability
- To improve the patient compliance
- To study the existence of the drug in the proniosome vesicles.
- To study the stability of drug loaded proniosome vesicles

4. Hypothesis

The prevalence of biochemical testosterone deficiency increases with age. This is partly due to decreasing testosterone levels associated with illness or debility but there is also convincing epidemiological data to show that serum free and total testosterone levels also fall with normal aging. The symptoms of aging include tiredness, lack of energy, reduced strength, frailty, loss of libido, decreased sexual performance depression and mood change. Men with hypogonadism experience similar symptoms. This raises the question of whether some symptoms of aging could be due to relative androgen deficiency. On the other hand, similarities between normal aging and the symptoms of mild androgen deficiency make the clinical diagnosis of hypogonadism in aging men more challenging. The oral bioavailability of testosterone conventional formulation is 3-10%. The bioavailability is limited by the presence of the hepatic first pass metabolism and poor solubility in the physiological media. In order to improve the bioavailability of testosterone the conventional tablets, the vescisular system the proniosome could be good candidates got oral delivery because they could not only improve the oral bioavailability of the testosterone. On the basis of previous work related to the application of proniosome in oral delivery, we can hypothesize that the proniosome would enhance the oral bioavailability of drug.

5. Research Methodologies

5.1 Preparation of Proniosome

Proniosome powders were prepared by using slurry method reported elsewhere. Accurately weighed amounts of mixture (250 mg) comprising of span 60 and cholesterol at various molar ratios and drug were dissolved in 20 mL of solvent mixture containing chloroform and methanol (2:1). The resultant solution was transferred into a 250 mL round bottom flask and Solid carrier (250 mg) was added to form slurry. The flask was attached to a rotary flash evaporator and the organic solvent was evaporated under reduced pressure at a temperature of 45 ± 2 °C. After ensuring the complete removalof solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature so as to obtain dry, free-flowing product. The obtained proniosome powders were stored in a tightly closed container at 4°C for further evaluation [58].

5.2 Optimization of proniosome using quality by design

A 3-factor, 3-level Box–Behnken design was used to optimize the proniosome formulation using STATISTICA version 11 (StatSoft, Inc., Tulsa, OK). The design is suitable for exploring quadratic response surfaces and constructing second order polynomial models. The design takes into consideration replicated centre points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest 12. The concentration of lipid (X1) concentration of drug (X2) and concentration of surfactant (X3) were selected as three independent formulation variables. Particle size (Y1) and % entrapment efficiency (EE) (Y2) were selected as dependent response variables. These high (þ1), medium (0) and low (1) levels were established from the preliminary experimentation and kept constant throughout the studies.

5.3 In vitro characterization of Drug loaded Proniosome

5.3.1 Micromeritic properties of proniosome powders

The flow properties of powder are vital in handling and processing operations. The flow properties were studied through measuring the Angle of repose, Carr’s compressibility

index and Hausner's ratio. The angle of repose was determined by using conventional fixed funnel method. The Carr's compressibility index and Hausner's ratio were calculated from the bulk and tapped density of the proniosome powders [58].

5.3.2 Particle size and zeta potential measurement

The volume average diameter and zeta potential of drug free or drug loaded Proniosome in dispersion were determined with Zetasizer after diluted 20 times with distilled water.

5.3.3 TEM examination

The morphological observation of drug loaded proniosome was performed by transmission electron microscopy (TEM). The samples were diluted for TEM observation.

5.3.4 DSC analysis

Differential scanning calorimetry (DSC) analysis was performed using DSC. For DSC measurement, 10 mg of powdered proniosome were put in aluminium pans. A scan rate of 10 °C/min was employed in the 20–80°C temperature range.

5.3.5 Drug entrapment efficiency determination

The drug loaded proniosome were dispersed in an 80 ml solution and surged vortically for 3 min to dissolve the free drugs. The resulting dispersions were centrifuged for 20 min at 25,000 rpm. The drug content in the supernatant after centrifugation was measured by UV method [58].

5.3.6 In vitro release assay

In vitro dissolution study of proniosomal powders and pure drug was performed using USP type II (paddle) apparatus in both simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) without enzyme. The volume of dissolution medium used was 900 ml and maintained at a temperature of 37 ± 0.5°C with paddle speed set at 50 rpm throughout the experiment. An aliquot of 5 ml was collected at predetermined time intervals up to 2 h and replaced with fresh dissolution medium to maintain constant volume. The samples were filtered by passing through 0.45 micron membrane filter and analyzed by UV [58].

5.3.7 Stability studies

The formulations containing capsule stored in glass vials covered with aluminium foil were kept at 25°C/60% RH and in refrigerator (4 ± 2°C) for a period of 90 days. At definite time intervals (0, 30, 60 and 90 days and 180 days), samples were withdrawn and hydrated with phosphate buffered saline pH (6.8) and observed for any sign of drug crystallization under optical microscope. Further the samples were also evaluated for % drug entrapment of Testosterone [58].

5.3.8 In vivo Pharmacokinetic study

SD rats (230–250 g body weight) were used for the pharmacokinetic study. The male SD rats were divided into three subgroups of five animals each. All animals were fasted overnight prior to the day of experiment with free access to water. Using an oral gavage, group 1 animals were administered with pure drug suspension and group 2 animals were administered with Proniosome formulation. Group 3 animals were administered with Market Preparation. Following oral administration, 0.3 ml of blood samples were collected from the cannula at 0.5, 1, 2, 4, 6, and 24 h and transferred into a heparinized Eppendorf tubes. A zero time sample was collected prior to administration of the dose. The collected blood samples were centrifuged at 13,000 rpm for 8 min at 4°C, and supernatant plasma was collected and stored at −80°C until analysis. Food was provided to the rats after 4 h following oral dosing. Calculate the bioavailability of all three formulations and other pharmacokinetic parameter. Animals were sacrificed after completion of the study according to the procedure defined in the IACUC protocol [60].

5.3.9 In vivo efficacy study [61]

To study the effect of the oral proniosome containing undeconate testosterone on the spermatogenesis. The 18 adult Wistar albino male rats were 8 weeks old and weighed 250 ± 10 g, they were obtained from the animal facility. Thereafter, the rats were randomly selected and divided into three groups:

- **Group 1: Control group (n = 6) for 28 days**
- **Group 2: 0.5 mg/kg dose of undeconate testosterone in arachis oil intramuscularly (n=6) for 28 days**

- **Group 3: 0.5 mg/kg dose of undeconate testosterone proniosome containing oral proniosome (n=6) for 28 days**
- **The control group just received 1cc of distilled water by gavage.**

Surgical procedure

On the twentieth eight day (at the end of the treatment period) the rats were killed with diethyl ether and the testes in the control and experimental groups were immediately removed. The weight of the testes in all study groups was recorded.

Epididymal sperm motility, viability, and counts, and sperm abnormality

Sperm from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin. After 5 min incubation at 37° C (with 5% CO2), the cauda epididymis sperm reserves were determined using the standard haemocytometric method, and sperm motility was analyzed by microscope at 10 field and reported as the mean of motile sperm according to WHO methods. The sperm abnormality was evaluated according to the standard method of Narayana. Briefly, smears of the sperm suspension were made on clean glass slides and stained with periodic acid-Schiff's reaction haematoxylin. The stained smears were observed under a light microscopic with 40× magnification. The sperms were classified into normal and abnormal. The total sperm abnormality was expressed as percentage incidence.

Serum total testosterone hormone

Serum concentration of total testosterone was measured by using a double antibody RIA kit from Immunotech Beckman Coulter, USA. The sensitivities of hormone detected per assay tube were 0.025 ng/ml.

Histology

The testis were fixed in 10% formalin and embedded in paraffin. Five-micron-thick sections were prepared and stained with haematoxylin and eosin (HE). The specimens were examined under an Olympus/3H light microscope.

6. Plan of work

6.1 Review of Literature

- Based on Drug
- Based on Formulation

6.2 Selection of drug and polymers

6.3 Preformulation studies of drug

- Organoleptic characterization
- Melting Point
- Determination of λ max and Standard curve
- UV method development and Validation
- Solubility of drug
- Partition coefficient of drug
- FTIR Spectra
- Drug-excipients compatibility study
- DSC (Drug, excipients and Physical mixture)
- XRD (Drug, excipients and Physical mixture)

6.4 Preparation of Drug loaded Proniosome

6.5 Optimization of Drug loaded Proniosome formulations using the QbD approach

6.6 Evaluation of Drug loaded Proniosome Percentage Yield

- Particle Size and Zeta Potential
- Drug entrapment and Percentage drug loading
- FTIR, DSC, XRD of optimized formulation
- TEM of optimized formulation
- In-Vitro drug release study
- In vitro drug release kinetic study

6.7 Stability study of the drug loaded proniosome

6.8 In vivo Pharmacokinetic study

6.9 In vivo efficacy study

7. References

1. Harman SM, Metter EJ, Tobin JD, et al. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. J Clin Endocrinol Metab. 2001;86:724–31.

2 Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. J Clin Endocrinol Metab. 2002;87:589–98.

3Campion JM, Maricic MJ. Osteoporosis in men. Am Fam Physician. 2003;67:1521–6.

4. Moffat SD, Zonderman AB, Metter EJ, et al. Free testosterone and risk for Alzheimer disease in older men. Neurology. 2004;62:188–93.

5Svartberg J, von Muhlen D, Schirmer H, et al. Association of endogenous testosterone with blood pressure and left ventricular mass in men. The Tromso Study. Eur J Endocrinol. 2004;150(1):65–71.

6. Barrett-Connor E. Lower endogenous androgen levels and dyslipidemia in men with non-insulin-dependent diabetes mellitus. Ann Intern Med. 1992;117:807–11.

7. Murphy S, Khaw KT, Cassidy A, et al. Sex hormones and bone mineral density in elderly men. Bone Miner. 1993;20:133–40.

8. Lorentzon M, Swanson C, Andersson N, et al. Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. J Bone Miner Res. 2005;20:1334–41

9. Svartberg J, von Muhlen D, Sundsfjord J, et al. Waist circumference and testosterone levels in community dwelling men. The Tromso study. Eur J Epidemiol. 2004;19:657–63

10. MacKay JD, Mensah GA. The atlas of heart disease and stroke. Geneva: WHO; 2004

11. Barrett-Connor E, Khaw KT. Endogenous sex hormones and cardiovascular disease in men. A prospective population-based study. Circulation. 1988;78:539–45.

12. Kapoor D, Goodwin E, Channer KS, et al. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. Eur J Endocrinol. 2006;154:899–906.

13. Ponholzer A, Temml C, Mock K, et al. Prevalence and risk factors for erectile dysfunction in 2869 men using a validated questionnaire. Eur Urol. 2005;47:80–5. discussion 85–6.

14. Shabsigh R, Anastasiadis AG. Erectile dysfunction. Annu Rev Med. 2003;54:153–68

15.Barrett-Connor E, Von Muhlen DG, Kritz-Silverstein D. Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. J Clin Endocrinol Metab. 1999;84:573–7

16 Nieschlag E, Swerdloff R, Behre HM, et al. Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, and EAU recommendations. Int J Androl. 2005;28:125–7

17. hurmeyer T, Wickings EJ, Freischem CW, et al. Saliva and serum testosterone following oral testosterone undecanoate administration in normal and hypogonadal men. Acta Endocrinol (Copenh) 1983;102:456–62

18. Nieschlag E, Cuppers HJ, Wiegelmann W, et al. Bioavailability and LH-suppressing effect of different testosterone preparations in normal and hypogonadal men. Horm Res. 1976;7:138–45

19. Schubert M, Minnemann T, Hubler D, et al. Intramuscular testosterone undecanoate: pharmacokinetic aspects of a novel testosterone formulation during long-term treatment of men with hypogonadism. J Clin Endocrinol Metab. 2004;89:5429–34

20. Handelsman DJ, Mackey MA, Howe C, et al. An analysis of testosterone implants for androgen replacement therapy. Clin Endocrinol (Oxf) 1997;47:311–16.

21. Behre HM, von Eckardstein S, Kliesch S, et al. Long-term substitution therapy of hypogonadal men with transscrotal testosterone over 7–10 years. Clin Endocrinol (Oxf) 1999;50:629–35

22. Parker S, Armitage M. Experience with transdermal testosterone replacement therapy for hypogonadal men. Clin Endocrinol (Oxf) 1999;50:57–62

23. Ross RJ, Jabbar A, Jones TH, et al. Pharmacokinetics and tolerability of a bioadhesive buccal testosterone tablet in hypogonadal men. Eur J Endocrinol. 2004;150:57–63

24. Song S, Tian B, Chen F, et al. Potentials of proniosomes for improving the oral bioavailability of poorly water-soluble drugs. Drug Dev Ind Pharm 2015;41:51–62.

25. Ahmad MZ, Akhter S, Mohsin N, et al. Transformation of curcumin from food additive to multifunctional medicine: nanotechnology bridging the gap. Curr Drug Discov Technol 2014;11:197–213.

26. Ahmad MZ, Alkahtani SA, Akhter S, et al. Progress in nanotechnology-based drug carrier in designing of curcumin nanomedicines for cancer therapy: current state-of-the-art. J Drug Target 2015.

27. Hu C, Rhodes DG. Proniosomes: a novel drug carrier preparation. Int J Pharm 1999;185:23–35.

28. Yasam VR, Jakki SL, Natarajan J, et al. A review on novel vesicular drug delivery: proniosomes. Drug Deliv 2014;21:243–249.

29. Wong M, Thompson TE. Aggregation of dipalmitoylphosphatidylcholine vesicles. Biochemistry 1982;21:4133–4139.

30. El-Laithy HM, Shoukry O, Mahran LG. Novel sugar esters proniosomes for transdermal delivery of vinpocetine: preclinical and clinical studies. Eur J Pharm Biopharm 2011;77:43–55.

31. Mokhtar M, Sammour OA, Hammad MA, et al. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int J Pharm 2008; 361:104–111.

32. Zidan AS, Mokhtar M. Multivariate optimization of formulation variables influencing flurbiprofen proniosomes characteristics. J Pharm Sci 2011;100:2212–2221.

33. Solanki AB, Parikh JR, Parikh RH. Formulation and optimization of piroxicam proniosomes by 3-factor, 3-level Box–Behnken design. AAPS PharmSciTech 2007;8:E86.

34. Veerareddy PR, Bobbala SK. Enhanced oral bioavailability of isradipine via proniosomal systems. Drug Dev Ind Pharm 2013;39: 909–917.

35. Akhilesh D, Prabhu P, Faishal G. Comparative study of carriers used in proniosomes. Int J Pharm Chem Sci 2012;4:307–314.

36. Yadav K, Yadav D, Saroha K, et al. Proniosomal gel: a provisicular approach for transdermal drug delivery. Der Pharm Lett 2010;2: 189–198.

37. Abd-Elbary A, El-laithy HM, Tadros MI. Sucrose stearate-based proniosome-derived niosomes for the nebulisable delivery of cromolyn sodium. Int J Pharm 2008;357:189–198.

38. Fang JY, Yu SY, Wu PC, et al. In-vitro skin permeation of estradiol from various proniosome formulations. Int J Pharm 2001;215:91–99.

39. Blazek-Welsh AI, Rhodes DG. Maltodextrin-based proniosomes. AAPS PharmSci 2001;3:E1.

40. Blazek-Welsh AI, Rhodes DG. SEM imaging predicts quality of niosomes from maltodextrin-based proniosomes. Pharm Res 2001;
18:656–661.

41. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int J Pharm 1994;105:1–6.

42. Ahmed S, El-Setouhy DA, El-Latif Badawi AA, El-Nabarawi MA. Provesicular granisetron hydrochloride buccal formulations: in vitro evaluation and preliminary investigation of in vivo performance. Eur J Pharm Sci. 2014;60:10–23.

43. Sahoo RK, Biswas N, Guha A, et al. Maltodextrin based proniosomes of nateglinide: bioavailability assessment. Int J Biol Macromol. 2014;69:430–434.

44. Akhtar M, Imam SS, Afroz Ahmad M, et al. Neuroprotective study of Nigella sativa-loaded oral provesicular lipid formulation: in vitro and ex vivo study. Drug Deliv. 2014;21:487–494.

45. https://en.wikipedia.org/wiki/Testosterone

46. Bain J, Schill W; Schwarzstein L, Treatment of Male Infertility. Springer Science & Business Media. 2012; 176–177.

47. Täuber U, Schröder K, Düsterberg B, Matthes H, Absolute bioavailability of testosterone after oral administration of testosterone-undecanoate and testosterone". EurJ Drug Metabol Pharmacok. 1986; 11 (2): 145–149.

48. Liang X, Hua Y, Liu Q, Li Z, Yu F, Gao J, Zhang H, Zheng A, Solid Self-Emulsifying Drug Delivery System (Solid SEDDS) for Testosterone Undecanoate: In Vitro and In Vivo Evaluation, Curr Drug Deliv. 2021; 18 (5): 620 – 633.

49. Shruthi PA, Pushpadass HA , Franklin MEE, Battula SB, N. Naik L, Resveratrol-loaded proniosomes: Formulation, characterization and fortification, LWT - Food Sci Technol. 2020; 134:110127.

50. Khudaira N, Agounia A , Elrayessb MA , Najlahc M , Younesd HM, Elhissi A, Letrozole-loaded nonionic surfactant vesicles prepared via a slurry-based proniosome technology: Formulation development and characterization, J Drug Del Sci Technol. 2020; 58:101721.

51. Hua Y, Li W, Cheng Z, Zhao Z, Yin X, Li Y, Zheng A, Solidification of Nanostructured Lipid Carriers Loaded Testosterone Undecanoate: In Vivo and In Vitro Study. Drug Res. 2018; 68(08):457–464.

52. Khatoon M, Shah KF, Din FU, Shah SU, Rehman AU, Dilawar N, Khan AN, (2017) Proniosomes derived niosomes: recent advancements in drug delivery and targeting, Drug Del. 2017; 24(1): 56-69.

53. Boddu M, Choppari V, Rapalli VK, Badam M (2017) Formulation and Evaluation of Proniosomes of Felodipine. Drug Des. 6: 154

54. Ahmad MZ, Mohammed AA, Ibrahim MM, Technology overview and drug delivery application of proniosome, Pharm Dev Technol. 2017; 22(3):302-311.

55. Shehata TM, Abdallah MH, Ibrahim MM. Proniosomal Oral Tablets for Controlled Delivery and Enhanced Pharmacokinetic Properties of Acemetacin. AAPS PharmSciTech.2014; 16(2): 375–383

56. Veerareddy PR, Bobbal SKR, Enhanced oral bioavailability of isradipine via proniosomal systems, Drug Dev Ind Pharma. 2012; 1–9.

57. Muchow M, Maincent P, Müller RH, Keck CM, Testosterone undecanoate – increase of oral bioavailability by nanostructured lipid carriers (NLC), J Pharma Technology Drug Res.2013, 2:4.

58. Gurrapu A, Jukanti R, Bobbala SR, Kanuganti S, Jeevana JB, Improved oral delivery of valsartan from maltodextrin based proniosome powders, Adv Pow Technol. 2012; 23 (5): 583-590.

59. Stanworth RD, Jones TH, Testosterone for the aging male; current evidence and recommended practice, Clin Interven Ag. 2008:3(1): 25–44.

60. Nekkanti V, Wang Z, Betageri GV, Pharmacokinetic Evaluation of Improved Oral Bioavailability of Valsartan: Proliposomes Versus Self-Nanoemulsifying Drug Delivery System, AAPS PharmSciTech, 2016; 17(4).

61. Khaki A, Fathiazad F, MNouri M, Khaki AA, Khamenehi HJ, Hamadeh M, Evaluation of androgenic activity of allium cepa on spermatogenesis in the rat, Folia Morphol.2009, 68(1):45–51.